To: BLA STN 125029
From: Gary Kikuchi
Through: Gibbes Johnson, DTP, Elizabeth Shores, DTP, Barry Cherney, Deputy Director DTP, Amy Rosenberg, Director DTP
Date: November 20, 2001

Immunogenicity Review of Xigris, Activated Protein C (Drotrecogin alfa, Lilly) for treatment of sepsis

I. Introduction/Administrative Issues:

Product: Xigris, or Activated Protein C (rhAPC, Drotrecogin alfa, Lilly)
Indication: treatment of patients with sepsis
Reviewers:

Chair: Gibbes Johnson
Additional product reviewers: Fred Mills (CMC), Gary Kikuchi (immunogenicity)
Pharm/Tox: Martin Green
Clinical Review Team: Linda Forsyth, Robert Lindblad, William Schwieterman (DCDTA branch chief)
CSO: Brad Glasscock

II. Summary

A. Product Overview: Recombinant human Activated Protein C (rhAPC) is a --- chain glycoprotein of approximate MW 45 Kd containing -- N-linked carbohydrate side chains. The product is produced upon transfection of human ---- --- ---- cells, which are grown in suspension culture. The final product is produced after in vitro enzymatic cleavage of the precursor molecule by thrombin. The mechanism of action of Activated Protein C is not known.

B. Assays for detection of anti-Activated Protein C antibodies. This review focuses on the immunogenicity of Activated Protein C as assessed by induction of an antibody response. Three antibody detection assays are described in the BLA, and the their attendant problems are discussed in detail in sections III, IV, and V below:

Level 1 assay: A sequential solid-phase chemiluminescent binding assay was used to screen serum samples for anti-APC ------ antibodies. ----------
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Level 2 assay: Samples that were positive in the Level 1 assay were tested in a confirmatory inhibition assay. -- -- -- -- -----------------

Level 3 assay: Samples positive in the Level 2 assay were further characterized using a two-step neutralization assay. -- -- in a standard APTT assay.

C. Key problems with antibody detection assays: (details in sections III-V)

1. Reporting and quantification methods:
   - Data are reported in -------- (--------------------------------). This method of quantification is difficult to assess.

2. Sensitivity:
   - The level 1 screening assay uses -----------------, so fails to detect immunoglobulin IgM, IgA, and IgG3 isotypes.

   - The presence of anti-APC antibodies of the IgM, IgA, or IgG3 isotype may inhibit the ability to detect anti-APC antibodies of other isotypes as non-protein A isotypes could block binding of protein A-binding isotypes to APC epitopes.

   - Antibody levels were monitored at two and four weeks after treatment with rhAPC. However, it is possible, if not probable, that antibodies will develop several weeks or even months after product administration. Additional studies monitoring long term development of antibodies are needed to assess the actual incidence of anti-APC antibodies.

     - The cutoff values for a positive response (------------------------------ -- --) are not supported by any data and thus appears to be set arbitrarily. Consequently, it is impossible to assess the incidence of anti-APC antibodies.

3. Specificity:
   - The specificity experiments were performed with a ---------- --------- monoclonal antibody. Because -------- --------- does not bind protein A well, the test cannot evaluate specificity. In addition, specificity experiments demonstrating inhibition with both soluble APC and non-APC products should be performed.

- The level 2 assay (which uses soluble APC to block and so represents a measure of assay specificity) fails to inhibit binding of sera samples from some patients that are deemed positive by the level 1 assay. Moreover, sera obtained from some placebo treated patients also appear as positive in the level 1 assay. Hence the there may be a high number of false positives detected in this assay. Consequently, it is difficult to assess the true incidence of anti-APC antibodies.

4. Incomplete validation of screening assays:

- Validation of the level 2 and level 3 (APTT) screening assays for sensitivity, ruggedness, reproducibility is incomplete

D. **Implications of antibodies to activated protein C.** In the integrated summary of safety, it is stated that 2/417 (0.5%) of patients developed antibodies to rhAPC in phase II and III clinical trials using the level 1 screening assay and confirmation by the level 2 inhibition assay. One patient in the phase III trial developed a thrombotic event and died of multiple organ dysfunction at study day 36, past the 28 day study period. Coagulation disorders are present in patients with hereditary deficiency in protein C, so antibodies that cross-react with endogenous APC might be imagined to induce a coagulation disorder that would produce thrombotic events. However, sepsis patients eligible to receive rhAPC may present clinically with coagulation disorders and disseminated intravascular coagulation. Therefore, any coagulation disorders induced by an effect of antibodies to rhAPC will be difficult to discern, if present very early in the course of treatment.

E. **Overall evaluation of immunogenicity assay and implications.** There are profound problems with the sensitivity, specificity, background (signal to noise ratio) and quantification of the Level 1 and Level 2 assays. **Because of these issues, it is not possible to assess the incidence of antibodies to APC.**

The product is intended for single administration by the IV route, which lowers the risk of an antibody response. If multiple infusions of rhAPC are considered for future indications, the possibility for development of antibodies to rhAPC will require further study.

F. **Recommendation:** Clinical review suggests that APC is efficacious for the indicated patient population. Consequently, the revisions to the immunogenicity assays are being requested in the form of post-marketing commitments, listed at the end of the review. As summarized above and discussed in detail in the following sections, the problems with the existing assays are profound such that refining and validating these assays are unlikely to prove useful. Consequently, Phase IV commitments (discussed at the end of the review) are being sought. Theses commitments focus on the design of a new assay with meaningful quantification, sensitivity, and specificity that will allow for more accurate

assessment of the incidence of anti-APC antibodies. The text of the post-marketing agreements is described in appendix A.

III. Detailed methods and validation – Level 1 Assay

A. Methods. ----------

Comments:

Several elements of this screening assay are unclear including;

- ----------
- ----------

---------- *is used as the detection methods.*

- ---------- *does not detect IgM, IgA, and IgG3 isotypes.*
- ----------

B. Data Reporting: Final data from the assay are reported in ---------- (--------). This value adjusts for day to day variations in the assay.
To calculate ---------, the following formula is used:

Raw ------ are the numbers provided by the ----------. The "assay factor" accounts for day-to-day variation of the assay. To calculate the "assay factor", the same ---- reference normal human serum and plasma samples are assayed every time the assay is run. The assay factor for each of these ---- reference samples is calculated as:

The overall "assay factor" for the assay is the mean of the assay factors for each of the --- reference normal human serum and plasma samples.

Comment:

Theoretically, this approach is similar to running a standard curve with a control sample for normalization. However, there are several major problems with this approach.

- *First, assay results should be presented in methods that clearly allow quantification of antibodies.*
- *Standard curves are based on serial dilutions of a positive control sample and have the advantage of being able assess assay validity as function of the linearity (r value of the curve). This advantage is not offered in the current assay.*
- *Finally, calculation of the "assay factor" only takes into account normal samples, which tend to have low values. Positive control ---------- antiserum, which have high values, are not used in calculation of the "assay factor".*

Additional justification is needed for these calculations. In the Comments to Sponsor, this is covered by a request for a new assay.

C. Elements of Level 1 assay validation:

In the BLA, information was provided to validate the sensitivity, inter-assay and intra assay variation, specificity, freeze-thaw stability, reproducibility with negative patient serum, and matrix effects of serum, EDTA, and heparin on the assay. Comments are provided below each of the validation studies.

1. **Sensitivity:** Sensitivity of the Level 1 antibody screening assay was evaluated using a positive control ---------- anti-human protein-C (----------.)

This ---------- antiserum was tested at ---------- dilutions. The

2. ------ of each of these dilutions was calculated and is provided in the following table:

----------	--------	----------
---------- ----------	---	--
----------	-----	------
----------	-----	-----
----------	---	----

The formula for the percent increase in ------ was not provided. However, it appears to have been calculated from the formula:

Comment on this analysis:

- *Of concern is the fact that the manufacturer of the ---------- antiserum indicates that the antiserum works at a ------- dilution in Western blots. However, in this assay, much higher concentrations of sera are required for positive results. This finding underscores the lack of assay sensitivity.*

- *The sponsor uses human sera ---- (----------) as a cut value for negativity. However, other normal human sera have a much higher value (up to --------- of ----). Application of this higher cut off value would indicate the rabbit serum has almost no activity in the assay. Hence, is not a useful measure of sensitivity.*

2. Intra-assay and inter-assay variation:

a. Inter assay variation. ---- normal human serum or plasma samples (----------) and ---------- dilutions of the positive control ------- antiserum were run each time the assay was performed, and are reported in the table below. As discussed above, the "assay factor" for each day is given by the formula:

In the table 2, the "assay factor" for each time the experiment was run (-- different times for the -- different samples) is calculated. . The -------- are calculated for each control -------- (negative human serum samples) and each of the --dilution of positive control -------- serum

Comment: The statistical analysis of "assay factor", control samples, and positive controls samples run at -- different times have adequate CVs, ranging from 5.2% to 13.8 %,. Consequently, the data support inter-assay reproducibility.

b. Intra-assay variation. Intra assay variation is addressed in table 3.

Positive control ------ serum were diluted ---------- and a pool of normal human serum were assayed for ----- replicates. CVs ranged from 3.7 to 9.3.

Comments: Taken together, the data shown in support of assay reproducibility indicate that both intra-assay variation and day-to-day variation are reasonable for this assay. However, operator variation, or results of the assays performed by different laboratory personnel, are not addressed.

3. Specificity: The sponsor addressed assay specificity by using two different commercially ---------- antibodies (spiked into normal serum) and measuring the --------. -------- is specific for the protein C activation peptide, and should be negative. ---------- is the positive control ---------- antibody, specific for activated protein C . The formula for the % difference is not provided, but is inferred to be:

% difference = [(-------- of positive control/-------- of N1) –1]x100

The results of this assay, demonstrate ------- (negative control) containing samples do not have -------- above background, whereas -------- (positive control) have ------- above

background with high % difference. The sponsor concludes that this demonstrates assay specificity.

Comments: There are three problems with this study.

- *The purported negative control antibody (-------) is an ------ antibody and has poor affinity for protein A. In contrast, --------- (specific for activated protein C) is a ----------, which binds protein A well. Hence, the results are meaningless.*

- *The ---------- monoclonal only gives strong positive results at a ----- dilution. However, the manufacturer of the antibody recommends a ---------- dilution for western blotting. This finding underscores the poor sensitivity of the assay.*

- *Specificity experiments should document the specific reactivity of the positive control antibodies for the product. This should be done by adding excess unlabeled product (or irrelevant protein) to inhibit specific binding of ------- labeled APC. Such experiments were not performed and assay specificity cannot be evaluated.*

4. **Freeze-thaw stability.** For assessment of freeze-thaw stability, the -------- positive control antibody underwent - and -- freeze-thaw samples and was assayed for activity. There did not appear to be any significant loss of activity between -- and -- freeze thaw cycles.

   Comment: While these experiments indicate the positive control ------- sera is not affected by repetitive freeze thaws, the stability of human serum or plasma samples was not assessed.

5. **Reproducibility (and matrix affects).** In order to validate the reproducibility of the assay, samples collected as serum, plasma collected in EDTA and plasma collected in heparin were studied. The means of these samples were calculated, but values greater than ---- were excluded from the calculation of the means. The overall normal range (excluding these outliers) is --- to --- --------.

   Comment: The reason for exclusion of ------- values over ----- from calculation of the means is not discussed, which is problematic. In addition, these data demonstrate that normal individuals can have quite high ------- values. The assay thus has problems with false positives, as discussed below. Finally, validation with negative samples is not informative. Samples that are negative are likely to remain below the cutoff value irrespective of matrix components and reproducibility of tests.

Matrix effects: In order to examine matrix effects, Serum, EDTA plasma, and heparin plasma from normal individuals (indicated by "id numbers" were spiked with —— anti-human protein C (———— at ———— and the resulting samples analyzed. The following tables indicate the results.

Table 7

Matrix Effects

Serum

	Amount of antibody added			% Increase	
Id	+0	+10%	+1%	+10%	+1%
51	47	355	65	655%	38%
324	223	376	231	69%	4%
399	29	506	59	1645%	103%
742	64	246	84	284%	31%
910	34	289	53	750%	53%
625	42	282	57	571%	36%

EDTA

	Amount of antibody added			% Increase	
Id	+0	+10%	+1%	+10%	+1%
51	46	343	66	646%	43%
324	197	420	220	113%	12%
399	28	504	57	1700%	104%
742	54	249	57	361%	6%
910	32	785	51	2353%	59%
625	43	308	61	616%	42%

Heparin

	Amount of antibody added			% Increase	
Id	+0	+10%	+1%	+10%	+1%
51	50	420	76	740%	52%
324	222	425	229	91%	3%
399	30	495	66	1550%	120%
742	70	214	69	206%	-1%
910	39	316	51	710%	31%
625	19	287	62	1411%	226%

Data from assays [illegible]
Added Antibody [illegible]

Comment: The results have little relevancy for the actual assay as citrated plasma, which was used to collect clinical samples, was not studied. Moreover, Activated protein C, as an anticoagulant, may be present in different levels in serum versus plasma. This activated protein C could potentially affect the assay results, and the potential variability due to this effect is not examined.

IV. Detailed Methods and validation – Level 2 (Inhibition assay):

The sponsor describes the Level 2 assay, or inhibition assay, as a totally different assay performed only on samples that are positive in the Level 1 assay. The principle of this assay is the same as the level 1 assay, except an attempt is made to ---------- of positive antibody samples with ----- (---------- APC) at the beginning step.

Comment: This assay uses the same methodology as the screening assay, hence all the same pitfalls apply. This assay is therefore not strictly a different assay. Indeed, this type of assay should have been used to confirm the specificity of the level one screening assay.

A. Methods

B. Elements of Level 2 assay validation:

Data from two experiments are provided to support validation of the inhibition assay. The first experiment addresses sensitivity of the standard curve, using control --------- antisera specific for APC. The second experiment addresses use of this assay on patient sera.

1. Sensitivity of the standard curve of the inhibition assay:

The assay employs positive --------- anti-serum (---------- described previously) to assess the ability of soluble APC to block ---------- of anti-APC antibodies.

Dilution ---------- antiserum	---------- ----------	Inhibition ----------	% Inhibition
----------	58	70	-20.7
---------	135	44	67.4
----------	546	60	89.0

This method uses a ---------- excess of ------- product to achieve inhibition, because ---------- APC is present at approximately -- microgram/tube and excess ---------- APC is present at -- micrograms/tube. A formula for percent inhibition is not provided, but this number appears to be calculated as

---

Comments: There are four problems.

· *This experiment is performed only twice, and there is a notation in the information provided that the data above indicate a failed experiment. Several successful experiments need to be performed.*

· *A calibration curve of ----- competitor needs to be performed to ensure that this amount (---fold excess) is optimal, and neither too much nor too little.*

· *The calculation of % inhibition does not take background into account. The sponsor seems to expect that complete inhibition would result in ---------- but this is an unlikely expectation. For example, if background is ----------, then complete inhibition of ----------t antiserum would yield* ***% inhibition = (135-30)/135 = 77.8%*** *using the formula above. However, it is not possible to get higher calculated % inhibition. The correct formula should take background into account. The current method yields an underestimate of percent inhibition.*

· *A non-specific protein inhibitor (negative control) was not tested. Hence the specificity of inhibition is not addressed.*

2. **Use of the inhibition assay on patient serum:** Four samples from two patients were run in the inhibition assay to determine if the inhibition assay performed appropriately on human serum. In this experiment, the results with the control ------- serum were as follows:

	----------	Inhibition --------	% inhibition
-------- antiserum -------	87	70	19.5
-------- antiserum -----	177	74	58.2
-------- antiserum ----	501	96	80.8

The control ---------- antisera performed appropriately in the results above.

In this same experiment, 4 human patient samples with elevated -------- were tested. It is not stated whether these patient samples meet the clinical criteria for a positive APC result (---fold increase over baseline coupled with ------- over -----).

Patient #	Visit	------- Accession #	Original result	--------- ------	Inhibition ------	% inhibition
------	1	----------	542	272	274	-0.7
------	2	----------	554	285	331	-16.1
------	1	----------	254	184	177	3.8
------	2	----------	282	189	190	-0.5

With these patient samples, the positive level 1 results cannot be inhibited by an excess of ------ product. The sponsors suggest that some non-specific ---------- components in the specimens interferes with the assay.

Comments: There are several problems with the Level 2 assay.

- *It is unclear if any of these samples are truly positive, hence it is unclear whether the assay fails to demonstrate specificity of human samples in this assay or if the uninhibited values with high ------- are simply false positive. In the latter case, inhibition would not be anticipated. Hence, this experiment is completely uninformative.*

- *Because the human patient samples cannot be inhibited, the specificity of the Level 1 assay is questionable.*

V. Detailed Methods and validation – Level 3 Assay:

B. Method. The Level 3 assay was performed on samples positive in the Level 2 assay. In the Integrated Summary of safety, it was mentioned that this assay was performed on samples from two patients, but the results of the Level 3 assay were negative for both of these samples.

With regard to assay methods, this assay is based on the concept of antibody inhibition of activated partial thromboplastin time (APTT), which is a clinical laboratory measure of coagulation dependent on presence of activated protein C. The APTT is expected to be shorter in the presence of inhibitory antibodies to APC. ----------

B. Elements of Level 3 Assay validation. Information is provided in the BLA regarding precision, accuracy, linear range, range of standard, normal range, and freeze-thaw stability of this assay.

The validation results are described as being based on the ---------- instrument with automated APTT reagent, (----------) lot # ----------. In the protocol it appears that ---------- ---------- (----------).

Comment regarding design: Protein G does not bind IgM, IgD, IgA, or IgE well, so the previous comment regarding specificity applies. ----------

1. Precision. The following information is provided regarding the precision of the level 3 assay:

	#1, No APC	#2, ------- rhAPC	#3, -------- plasma-derived APC	#4, ------- rhAPC + control -----	#5, ------- rh APC + --- -- ---------- anti APC
N	9	12	6	3 3	4 4
Mean (sec)	35.5	89.9	96.4	78.2 85.8	51.6 53.4
STD	1.2	4.0	8.3	2.2 4.7	2.8 5.1
CV	3.4	4.4	8.6	2.9 5.5	5.4 9.6

In this assay, the ---------- monoclonal appears to inhibit the APTT.

Comments: The concentration of the --- antibody is not given, and a dilution curve is not run. How the concentration of the ---------- relates to the concentration of antibodies in clinical samples is not clear. Without clear information regarding these antibody concentrations, the utility of this APTT assay is not clear.

2. **Accuracy, linear range, and range of standard.** For accuracy, it is stated that there was no known assayed material for this assay. For linear range, it was stated that samples are run undiluted. For range of standard, it was stated that this was not applicable, because there is no standard curve run in this assay.

3. **Normal Range.** It was stated that a normal range for this assay was established using -- individual samples (laboratory volunteers). The range was ---------- -- seconds with a mean of ----- seconds for patient ---- incubated with ------ APC under standard buffering conditions (----------.) The range was ---------- seconds with a mean of ----- seconds for patient ----- incubated with --- nM plasma-derived human activated protein C under the same buffering conditions.

4. **Freeze-thaw stability.** --- normal plasma samples from laboratory volunteers were spiked with ----------l antibody against human Protein C. Each was assay fresh and after --- freeze-thaws. Patient ---- was incubated with ------- rhAPC in the standard buffer indicated above.

Subject initials	-----	-----
--- freeze thaws	---- seconds	---- seconds
--- freeze thaws	---- seconds	---- seconds

Comments: Additional information regarding sensitivity, specificity, reproducibility, robustness, and ruggedness needs to be provided before this assay can be considered a truly validated assay. Finally, the ability of this assay to detect human antibodies to activated protein C needs to be validated.

VI. Overall clinical results and implications of immunogenicity assays

A. Patient Population – Indication, dose, route and frequency of administration: Xigris is proposed for use in treating sepsis associated with organ dysfunction. The proposed dosage is 24 micrograms/kg/hour intravenously for a total duration of 96 hours. A 70 kg patient would therefore receive a total dosage of approximately 161 milligrams, This is a high dose of product, which increases the probability of immunogenicity despite the fact that normal individuals express this protein so are expected to be tolerant. However, because only a single administration is intended, and it is delivered IV, the probability of observing immunogenicity is reduced. The effect of sepsis with massive cytokine release on immunogenicity is not known.

B. Timing of antibody sampling and cutoff. In the phase II/III trial -------, patients were tested for antibodies 14 days after the administration of product. In the phase III study ----------, 317 patients had samples for antibody testing collected at baseline and at least one sample collected on or after study day 12. Some patients were tested at 28 days.

Comment: Due to the 28 day study design, antibodies were tested at two and four weeks. However, this is an extremely short time to test for antibodies; in humans, -------- antibodies may not be detectable until six weeks to several months post-exposure. This concern is particularly applicable as patients may be immunoincompetent while septic. The short time course also makes assaying for IgM antibodies more essential.

In the integrated summary of safety, it is stated that the criteria for considering a sample positive is a ----fold or greater increase over baseline coupled with an ------ value for the sample of greater than or equal to ------.

Comment: There is no discussion of why these criteria are appropriate. In general, criteria for considering a sample positive should be based on tests with a large number of negative control human sera and a cut off value derived from a multiple of the standard deviation derived from such test. This multiple of the standard deviation should be determined based on a defined confidence limit.

A discussion of the Level 2 cutoff (stated in the Integrated Summary of safety to be ----% or more inhibition) in terms of the Level 2 results is provided below.

Results of immunogenicity assays by trial

1. Phase I/IB results: Anti-APC antibody results were monitored using the assays mentioned above in 104 patients in phase I/IB studies. 90/104 of these patients were exposed to rhAPC more than once. 19/104 of these patients received rhAPC four to six times. It was stated in the Integrated Summary of Safety that none of these subjects had anti-APC responses. The following table summarizes the results of the phase I/IB repeat dosing studies. Given in the following table are the number of rhAPC doses and the range of total amount of rhAPC per dose (in the suggested label this is given over 96 hours.)

Table ISS.12.2. Number of Exposures to rhAPC for Subjects in Phase 1/1B Studies Who Were Tested for Anti-APC Antibodies

Number of Unique Subjects N=105	Number of rhAPC Doses Received	Dose Range of rhAPC (µg/kg)
14	1	23 to 300
53[a]	2	36 to 1440
19	3	0.17 to 600
8	4	0.17 to 1270
5	5	0.73 to 1100
6	6	36 to 1300

Abbreviation: N = the total number of unique subjects in the Phase1/1B studies who were exposed to rhAPC and where immune response was monitored.

[a] Subject —— was exposed to rhAPC twice in Study —— however; antibody data for this subject are missing.

Source: Phase 1 Dose Summary – Final.doc and Repeat Dose Range.xls created by ——

Comment: Note that most of these patients were treated with doses less than the total suggested dose of 2304 micrograms/kg (24 micrograms/kg/hour given over 96 hours.) Therefore it cannot be concluded that repeat dosing at the labeled dose might induce an immune response at a rate greater than that observed after a single dose in phase III clinical trials. Because of these issues, references to lack of an antibody response after repeat dosing should be eliminated from the labeling.

2. Phase II/III results: In the phase II study ——, 90 septic patients were treated with rhAPC and patients were monitored at 14 days following the start of rhAPC infusion. 53 patients provided post-infusion serum and plasma samples for anti-APC antibody testing. Two of these patients, ——. infused with APC at 24 micrograms/kg/hr for 48 hours, and —— infused with APC at 12 micrograms/kg/hr for 48 hours were positive for anti-APC antibodies by Level 1 testing. These results are summarized in the table provided below. A follow-up sample from patient —— approximately one year after exposure was negative by Level 1 testing.

3. In the phase III study [redacted], 317 patients had samples for antibody testing collected at baseline and at least one sample colleted on or after study day 12.

The [redacted] clinical report states that only one patient, [redacted], in the pivotal phase III study of XIGRIS, developed antibodies to rhAPC as determined by positive results from both the Level 1 and level 2 assays. This patient developed a thrombotic event and death occurred of multiple organ dysfunction at day 36, outside the 28 day study period.

The following table from the Integrated Summary of Safety summarizes the results from all phase II and phase III studies:

Table ISS.12.3. Anti-APC Antibody Data for rhAPC-Treated Patients with Positive Level 1 Testing

	Level 1 Result:		Level 2 Result:		Level 3 Result:
Patient #	Fold Increase Over Baseline Value	[redacted] Units for Day 14 or Day 28 Sample	% Inhibition	Anti-APC antibody Response	Anti-APC Neutralizing Antibody
Phase 2					
[redacted]	7.9	388	53.0	Positive	Negative
[redacted]	2.2	142	27.2	Negative	Not applicable
Phase 3					
[redacted]	4.20	189	36	Negative	Not applicable
[redacted]	8.1	171	49.1	Positive	Negative
[redacted]	4.4	128	7.8	Negative	Not applicable

Abbreviation: [redacted] Phase 2 = Study [redacted] Phase 3 = Study

Source: [redacted] Report Analysis Plan, and data from [redacted] (ANTIBODY).

Comment: It is not clear why the inhibition cutoff was set at [redacted]. Moreover, the data presented are so completely manipulated (no raw data for baseline or inhibition [redacted]), it is not possible to discern if meaningful inhibition (or lack there of) occurred with patient samples. Patients [redacted] and [redacted] had 27% and 36% inhibition respectively. It is not clear why the inhibition cutoff was set at [redacted] from the information on assay validation for the level 2 assay.

To verify the above information the results from the ---------- phase III study provided in the SAS transport file were re-analyzed. The following table describes patients in the ------ that appear to be positive in CBER analysis of results of the level 1 screening assay provided in the SAS transport file according to the criteria provided above:

Incidence of antibody positive patients by Level 1 screening assay

#	Therapy	Site	Patient	Baseline ------	Day 14 ------	Day 28 ------	Day 14 Positive	Day 28 Positive
1	Placebo	----	------	22	38	164		+
2	Placebo	----	------	31	147	171	+	+
3	Placebo	----	------	145	390	306	+	+
4	Placebo	----	------	70	88	170		+
5	Placebo	----	------	34	159	169	+	+
6	Placebo	----	------	58	92	132		+
7	Placebo	----	------	28	255	ND	+	
8	APC	----	------	45	189	ND	+	
9	APC	----	------	85	173	126	+	
10	APC	----	------	24	213	98	+	
11	APC	----	------	21	37	171		+
12	APC	----	------	29	123	128		+

Comment: From this table, several conclusions can be made. First, until the Level 2 assay is properly validated, and justification for the ---% cutoff provided, the sponsor should report the incidence of patients with antibodies to product using the Level 1 screening assay. The incidence should be verified by the sponsor. If an incidence rate is required in the labeling, a rate of 5/317 or 1.5% in phase III trials, should be reported. However, until a fully validated, specific, sensitive assay with reasonable cut off values is designed, the true incidence of anti-APC antibody generation cannot be accurately assessed.

Indeed, there appears to be a significant incidence of antibody responses in patients that received placebo rather than product. The reason for this is not clear. This could be due to a natural response to elevation of endogenous APC present during the process of sepsis. Of more concern is the possibility that this is due to lack of assay specificity or an unexplained false positive rate.

2. **Concerns regarding potential immunoreactivity of activated protein C.** Coagulation disorders have been shown to be present in patients with hereditary deficiency in protein C (see Sakata et al., Thrombosis Research (1999) 94: 69 and Kario et al., Am J. Hypertension (2001) 14: 818). A similar clinical picture might be imagined to be present in patients with neutralizing antibodies to activated protein C.

Because thrombosis, potentially associated with antibodies to rhAPC was observed in one patient, heightened surveillance of antibody responses is warranted. The labeling proposed by the sponsor will need revision; appropriate post-marketing studies are recommended and are discussed below.

VII. Labeling.

1. Labeling proposed in BLA:

"Immunogenicity

In patients with severe sepsis, the formation of anti-activated protein C antibodies was uncommon (<1%) after a single course of therapy. These antibodies were not capable of neutralizing the effect of activated protein C on the APTT assay. XIGRIS has not been readministered to patients with severe sepsis. No anti-activated protein C antibody formation was detected in healthy subjects, even after repeat administration up to 6 times."

As discussed below, the first two sentences will need to be modified, because the sponsor has not demonstrated that the Level 1 assay has been qualified to a sensitivity level appropriate to detect antibodies. The APTT neutralization assay has not been validated, so this sentence will need to be removed. Statements regarding healthy subjects will need to be analyzed in light of the data. Standard boilerplate language with a disclaimer on the assay sensitivity will need to be inserted into the label.

2. Revised and agreed upon labeling as of November 9, 2001:

Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The incidence of antibody development in patients receiving Xigris has not been adequately determined, as the assay sensitivity is inadequate to reliably detect all potential antibody responses. One patient in the phase 2 trial developed antibodies to Xigris without clinical sequelae. One patient in the phase 3 trial who developed antibodies to Xigris developed superficial and deep vein thrombi during the study, and died of multi-organ failure on day 36 post-treatment but the relationship of this event to antibody is not clear.

Xigris has not been readministered to patients with severe sepsis.

Comments to Sponsor (based on information provided in BLA):

The following comments were discussed with the sponsor during a telecon on October 12, 2001:

We find that the current anti-APC antibody screening assays are insensitive, have a high background (noise to signal ratio), and are poorly quantifiable. Therefore, it is not possible at this time to assess the incidence of antibodies to APC. Consequently, the following post-marketing commitments are being requested.

Post Marketing Agreements

1. We note that your current immunogenicity assays (levels 1-3) lack sensitivity and are poorly quantifiable. Moreover, the issue of assay specificity is incompletely assessed.

    a. Please develop an assay that will detect all antibody isotypes. The use of Proteins A/G are discouraged, as they do not allow detection of all immunoglobulin isotypes, and non-Protein binding isotypes may actually inhibit the interaction of Proteins A/G-binding antibodies with APC. Please keep these considerations in mind in the design of an improved assay.

    b. Samples from some placebo treated patients appeared to have positive values according to your current assay. These values may reflect serum/plasma binding components capable of binding in the assay or natural antibody to APC which are elevated in septic patients. Using the improved assay, please completely evaluate the differences in reactivity among sera from non-septic patients, placebo treated septic patients, and APC-treated septic patients.

    c. Consequent to the issue discussed above, please provide a complete justification for cutoff values used to define positive vs. negative samples in the new assay.

    d. Please be aware that use of plasma may be problematic in assays for APC-binding antibodies as plasma can contain a larger variety of proteins than sera. Please consider this possibility when obtaining samples from patients.

    e. Please provide all details on the assay to CBER, including a complete description and source identification of all reagents used in the assay.

f. Please completely validate the assay for limits of detection, specificity, precision (reproducibility), and ruggedness.

g. Milestones for submission of assay study and validation reports and annual reports should be submitted prior to product approval.

2. Please develop and validate an assay that will allow for the assessment of APC neutralizing antibodies. Please keep in mind the issues regarding assay sensitivity, specificity, quantification, and reproducibility discussed in item #1.

3. Please be aware that development of human antibodies to activated protein C may take longer than the period studied during the clinical trial. Please obtain samples from patients at time points beyond 28 days after initiation of treatment with APC.

Review Appendix A

Post-marketing agreements proposed by Lilly on November 16, 2001:

1. To develop and evaluate an improved immunogenicity screening assay for detecting antibodies to drotrecogin alfa (activated). The design (with validation plan) and the results of your evaluation and validation data for this improved screening assay will be submitted by November 30, 2001 and April 1, 2002, respectively.
2. To provide more complete validation data for the existing level 3 immunogenicity neutralizing antibody assay by April 1, 2002.
3. To analyze, using the improved and validated immunogenicity screening assay, archived serum samples on patients from the phase 3 trial (----------) with both baseline and post-baseline samples from both placebo and drotrecogin alfa (activated) treatment groups. The results, with revised labeling if applicable, will be submitted by August 1, 2002. If antibodies to drotrecogin alfa (activated) are detected, Lilly will submit data establishing whether these antibodies neutralize the anticoagulant (APTT) activity of activated protein C by the level 3 immunogenicity assay.
4. To monitor the immunogenicity response to drotrecogin alfa (activated) treatment in patients with severe sepsis post-28 days in the current on-going open-label study ----------. The addendum for this protocol will be submitted on December 1, 2001. The results of the immunogenicity assessment will be submitted as part of the final study report in June 2003.
5. To collect additional samples for immunogenicity testing from the proposed phase III low APACHE score study. This will include samples taken pre-exposure and at relevant post-exposure time points. The number of samples to be collected and analyzed will be determined in consultation with the Agency after reviewing the data from the re-analysis of the phase 3 trial (----------) samples submitted August 2002.